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SOD1^{G93A} transgenic mouse CD4^{+} T cells mediate neuroprotection after facial nerve axotomy when removed from a suppressive peripheral microenvironment

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Abstract

Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease involving motoneuron (MN) axonal withdrawal and cell death. Previously, we established that facial MN (FMN) survival levels in the SOD1^{G93A} transgenic mouse model of ALS are reduced and nerve regeneration is delayed, similar to immunodeficient RAG2$^{-/-}$ mice, after facial nerve axotomy. The objective of this study was to examine the functionality of SOD1^{G93A} splenic microenvironment, focusing on CD4^{+} T cells, with regard to defects in immune-mediated neuroprotection of injured MN. We utilized the RAG2$^{-/-}$ and SOD1^{G93A} mouse models, along with the facial nerve axotomy paradigm and a variety of cellular adoptive transfers, to assess immune-mediated neuroprotection of FMN survival levels. We determined that adoptively transferred SOD1^{G93A} unfractionated splenocytes into RAG2$^{-/-}$ mice were unable to support FMN survival after axotomy, but that adoptive transfer of isolated SOD1^{G93A} CD4^{+} T cells could. Although WT unfractionated splenocytes adoptively transferred into SOD1G93A mice were able to maintain FMN survival levels, WT CD4^{+} T cells alone could not. Importantly, these results suggest that SOD1^{G93A} CD4^{+} T cells retain neuroprotective functionality when removed from a dysfunctional SOD1^{G93A} peripheral splenic microenvironment. These results also indicate that the SOD1^{G93A} central nervous system

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microenvironment is able to re-activate $CD4^+$ T cells for immune-mediated neuroprotection when a permissive peripheral microenvironment exists. We hypothesize that dysfunctional $SOD1^{G93A}$ peripheral splenic microenvironment may compromise neuroprotective $CD4^+$ T cell activation and/or differentiation, which, in turn, results in impaired immune-mediated neuroprotection for MN survival after peripheral axotomy in $SOD1^{G93A}$ mice.

Keywords

motoneuron; T cell; APC; axotomy; SOD1; ALS; immune

Introduction

In previous studies, we have established an immune-mediated model of endogenous neuroprotection following facial nerve axotomy in wild-type (WT) and immunodeficient recombinase activating gene-2 knock-out ($RAG2^{-/-}$) mice lacking functionally mature B and T cells, but intrinsically maintaining antigen presentation by MHC class II-expressing peripheral antigen-presenting cells (APC; Serpe et al., 1999; Serpe et al., 2003). Key to immune-mediated neuroprotection after axotomy is the generation of neuroprotective $CD4^+$ T cells that are antigen-speifc and require: 1) initial activation peripherally, *via* interaction with MHC class II-expressing APC, and 2) re-activation centrally, *via* interaction with MHC class II-expressing microglia (Byram et al., 2004).

Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease resulting in motoneuron degeneration and accompanied by neuroinflammation involving reactive microglia and astrocytes centrally and immune activation peripherally (Appel et al., 2010; Philips and Robberecht, 2011). The most widely used transgenic mouse model of ALS, involving the overexpression of human mutant superoxide dismutase-1 ($SOD1^{G93A}$), develops disease pathology similar to that in familial and sporadic ALS patients (Rosen et al., 1993; Gurney, 1994; Gurney et al., 1994). An axonal die-back response precedes MN cell death in SOD1 mice (Kennel et al., 1996; Fischer et al., 2004; Hegedus et al., 2007), resulting in a cascade of events similar to that observed in WT mice after peripheral nerve injury. Specifically, axonal degeneration, denervated neuromuscular junctions, afferent presynaptic stripping surrounding MN cell bodies in CNS, immune cell activation peripherally, and glial activation centrally are responses that occur both as a result of axonal die-back in ALS and peripheral nerve injury (Moran and Graeber, 2004; Jones et al., 2005; Zang et al., 2005; Chiu et al., 2009; Jinno and Yamada, 2011).

$SOD1^{G93A}$ mice demonstrate significantly increased FMN cell death following either a facial nerve transection or crush axotomy, relative to WT (Mesnard et al., 2011; Mesnard et al., 2013). Interestingly, while axotomized $SOD1^{G93A}$ FMN respond with a pro-regenerative response similar to WT, a dysregulated response to axotomy exists in the microenvironment surrounding the $SOD1^{G93A}$ FMN cell bodies (Mesnard et al., 2011). Importantly, target disconnection via disease or facial nerve axotomy in $SOD1^{G93A}$ mice results in comparable motoneuron- and glial-specific molecular changes within the facial nucleus (Haulcomb et al., 2014). Furthermore, $SOD1^{G93A}$ FMN exhibit a delayed functional recovery response to

facial nerve crush axotomy, relative to WT mice (Mesnard et al., 2013), that resembles the delayed functional recovery response of FMN in immunodeficient mice following facial nerve crush (Serpe et al., 2002). Therefore, both peripheral and central immune cell irregularities appear to impact $SOD1^{G93A}$ FMN survival and functionality after facial nerve axotomy.

The main objective of the current study was to begin to define whether an immune defect in $SOD1^{G93A}$ $CD4^+$ T cell development, activation, or re-activation is associated with the increased susceptibility of SOD1 FMN to axotomy-induced cell death or the defect lies within the previously identified central glial response (Mesnard et al., 2011). Through a variety of adoptive transfer experiments utilizing $SOD1^{G93A}$ and $RAG2^{-/-}$ mice, our results suggest that a defective $SOD1^{G93A}$ peripheral microenvironment and/or response, rather than a defect in the $CD4^+$ T cell itself, may underscore the impaired immune-mediated neuroprotection required for motoneuron survival and regeneration.

Materials & Methods

Animals

Female, C57Bl/6 wild-type (WT) and transgenic SOD1 ($SOD1^{G93A}$) were obtained from Jackson, and recombination activating-2 gene knock-out ($RAG2^{-/-}$) from Taconic, at 6 weeks of age and permitted 1 week to acclimate prior to experimental manipulation. The mice were provided autoclaved pellets and water ad libitum, and housed under a 12 h light/dark cycle in microisolater cages contained within a laminar flow system to maintain a pathogen-free environment.

Cellular adoptive transfers

Cellular adoptive transfers were completed at 7 weeks of age and 1 week prior to undergoing facial nerve axotomy. Spleens were removed from WT or $SOD1^{G93A}$ mice, and the splenocytes were isolated as previously described by our laboratory (Serpe et al., 1999; Byram et al., 2003; Serpe et al., 2003). Specifically, WT or $SOD1^{G93A}$ splenocytes were collected for adoptive transfer at a concentration of 50×10^6 splenocytes/100 μL PBS *via* tail vein injection per animal. Naïve WT or $SOD1^{G93A}$ $CD4^+$ T cells were isolated from the splenocyte samples *via* autoMACS magnetic cell sorting for adoptive transfer at a concentration of 5×10^6 $CD4^+$ T cells/100 μL PBS per animal. Axotomy-activated $CD4^+$ T cells (Byram et al., 2004) were isolated from WT or $SOD1^{G93A}$ spleens 3 days after facial nerve axotomy *via* autoMACS. Ten specific groups (N = 3-6/group) were utilized for FMN survival analyses, including WT, $SOD1^{G93A}$ $RAG2^{-/-}$, $SOD1^{G93A}$ + WT splenocytes, $RAG2^{-/-}$ + $SOD1^{G93A}$ splenocytes, $RAG2^{-/-}$ + WT $CD4^+$ T cells, $RAG2^{-/-}$ + $SOD1^{G93A}$ $CD4^+$ T cells, $SOD1^{G93A}$ + WT $CD4^+$ T cells, $SOD1^{G93A}$ + WT CD4-depleted splenocytes, and $SOD1^{G93A}$ + Axot WT $CD4^+$ T cells. All $SOD1^{G93A}$ mice were utilized by 12 weeks of age, and pre-symptomatic.

Facial nerve axotomy

A right facial nerve transection axotomy was performed in mice at 8 weeks of age. All surgical procedures were completed in accordance with National Institutes of Health

guidelines on the care and use of laboratory animals for research purposes. Using aseptic techniques, mice were anesthetized with 3% isoflurane and maintained at 1.5%, and the right facial nerve was exposed and completely transected at its exit from the stylomastoid foramen (Jones and LaVelle, 1985; Serpe et al., 1999). The proximal and distal facial nerve stumps were manually pushed away from each other in order to prevent reconnection. The left facial nerve remained intact, leaving the left facial nucleus to serve as an internal control for comparison purposes.

Facial motoneuron (FMN) counts

For each experiment, FMN survival levels were assessed at four weeks following facial nerve transection axotomy, where surviving FMN were counted in the ipsilateral facial nucleus and compared to the contralateral uninjured (control) facial nucleus. At 4 weeks post-axotomy, 25 μm cryosections were collected throughout the rostrocaudal extent of the facial motor nucleus (Mesnard et al., 2011). FMN were counted in thionin-stained tissue sections, and after application of the Abercrombie correction factor, results were presented as the average percent of FMN survival ± the standard error of the mean (SEM), as described in detail (Serpe et al., 1999; Mesnard et al., 2011). Representative photomicrographs of facial motor nuclei were obtained with an Olympus microscope and Image-Pro software. Statistical analysis was accomplished using a one-way ANOVA, followed by the Student-Newman-Keuls post-hoc multiple comparison test, with significance at $p < 0.05$.

Results

Significant FMN cell loss in SOD1^{G93A} and RAG2$^{-/-}$ mice after axotomy

Compared to the uninjured facial nucleus in WT mice (92 neurons ± 2; Figure 1A), baseline FMN counts were not altered in transgenic mice containing overexpression of the human mutant *SOD1*^{G93A} gene (90 neurons ± 2; Figure 1B) or deletion of the *RAG2* gene (90 neurons ± 3; Figure 1C) at 12 weeks of age (*quantitative data not shown*). However, at 4 weeks post-axotomy, FMN survival levels in the axotomized facial nucleus of WT mice (84% ± 2.0; Figures 1D and 2) were significantly higher compared to both SOD1^{G93A} (68% ± 1.0; Figures 1E and 2) and RAG2$^{-/-}$ mice in (57% ± 2.5; Figures 1F and 2), relative to the respective uninjured facial nuclei.

WT splenocytes adoptively transferred into SOD1^{G93A} mice maintain axotomized FMN survival levels

To determine whether the increased axotomy-induced cell death in SOD1^{G93A} mice could be prevented with the addition of naïve peripheral immune cells, WT unfractionated splenocytes were adoptively transferred *via* tail vein into SOD1^{G93A} mice prior to axotomy. At 4 weeks post-axotomy, adoptive transfer of WT splenocytes into SOD1^{G93A} mice (SOD1^{G93A} + WT splenocytes) prior to axotomy resulted in maintenance of FMN survival levels at 76% ± 1.8 in the axotomized facial nucleus, relative to the uninjured facial nucleus (Figures 1G and 2). The FMN survival levels in the SOD1^{G93A} + WT splenocytes group were similar to the levels observed in WT, and significantly elevated compared to SOD1^{G93A} mice without adoptive transfer. These results suggest that a potential defect

exists within the intrinsic peripheral immune cells of SOD1^{G93A} mice, and that the introduction of extrinsic WT peripheral immune cells into SOD1^{G93A} mice prior to axotomy prevents the axotomy-induced cell death.

SOD1^{G93A} splenocytes adoptively transferred into RAG2$^{-/-}$ mice unable to sustain WT levels of axotomized FMN

To determine whether SOD1^{G93A} splenocytes were capable of restoring WT FMN survival levels in RAG2$^{-/-}$ mice, as observed with WT unfractionated splenocytes (Serpe et al., 2000), unfractionated splenocytes from SOD1^{G93A} mice were adoptively transferred into RAG2$^{-/-}$ mice prior to axotomy. At 4 weeks post-axotomy, FMN survival levels in the axotomized facial nucleus of RAG2$^{-/-}$ mice that received an adoptive transfer of SOD1^{G93A} unfractionated splenocytes (RAG2$^{-/-}$ + SOD1^{G93A} splenocytes) were 60% ± 3.4, relative to the uninjured facial nucleus (Figures 1H and 2), comparable to FMN survival levels in the RAG2$^{-/-}$ group and significantly reduced relative to WT group. Thus, splenocytes from SOD1^{G93A} mice were not capable of the immune-mediated neuroprotection required to maintain RAG2$^{-/-}$ FMN survival levels.

SOD1^{G93A} CD4^{+} T cells adoptively transferred into RAG2$^{-/-}$ mice as effective as WT CD4^{+} T cells in restoring FMN survival levels after axotomy

In previous studies, B, CD4^{+} T, CD8^{+} T, CD4^{+}CD25^{+} Treg, NKT, and NK cellular populations were separately isolated from WT splenocytes and adoptively transferred into RAG2$^{-/-}$ mice prior to axotomy (Serpe et al., 2000; Byram et al., 2003; Serpe et al., 2003; DeBoy et al., 2006a; Deboy et al., 2006b). Only CD4^{+} T cells were found to provide the necessary immune-mediated support required to maintain RAG2$^{-/-}$ FMN survival levels to those of WT after axotomy (Serpe et al., 2003). The immune-mediated neuroprotective ability of SOD1^{G93A} CD4^{+} T cells was compared to WT CD4^{+} T cells *via* adoptive transfer of the respective CD4^{+} T cells into RAG2$^{-/-}$ mice prior to axotomy. At 4 weeks post-axotomy, FMN survival levels in the axotomized facial nucleus of RAG2$^{-/-}$mice that received an adoptive transfer of WT CD4^{+} T cells (RAG2$^{-/-}$ + WT CD4^{+} T cells) were 82% ± 2.1 (Figures 1I and 2) and in the axotomized facial nucleus of RAG2$^{-/-}$ mice that received an adoptive transfer of SOD1^{G93A} CD4^{+} T cells (RAG2$^{-/-}$ + SOD1^{G93A} CD4^{+} T cells) were 78% ± 1.9 (Figures 1J and 2), relative to the uninjured facial nucleus. FMN survival levels in the RAG2$^{-/-}$ + WT CD4^{+} T cells and the RAG2$^{-/-}$ + SOD1^{G93A} CD4^{+} T cells groups were significantly increased compared to the RAG2$^{-/-}$ group, and comparable to the WT group. These results indicate that SOD1^{G93A} CD4^{+} T cells are as effective as WT cells in mediating neuroprotection of axotomized FMN, when placed in a non-SOD1^{G93A} peripheral microenvironment (*i.e.*, RAG2$^{-/-}$), and support the concept that other cells in the SOD1^{G93A} central and/or peripheral immune compartment(s) may prevent neuroprotective SOD1^{G93A} CD4^{+} T cell development after axotomy.

Increased naïve CD4^{+} T cells, from WT mice, adoptively transferred to SOD1^{G93A} mice unable to support WT levels of axotomized FMN

To investigate whether the addition of isolate WT CD4^{+} T cells could prevent the increased susceptibility of SOD1^{G93A} FMN to axotomy-induced cell death, WT CD4^{+} T cells were

adoptively transferred into SOD1^{G93A} mice prior to axotomy. Thus, the number of CD4^{+} T cells in the intrinsic peripheral immune compartment of SOD1^{G93A} mice was increased in an attempt to determine if this would prevent the susceptibility of SOD1^{G93A} FMN to axotomy-induced cell death. At 4 weeks post-axotomy, FMN survival levels in the axotomized facial nucleus of SOD1^{G93A} mice that received an adoptive transfer of WT CD4^{+} T cells (SOD1^{G93A} + WT CD4^{+} T cells) were 72% ± 2.8, relative to the uninjured facial nucleus (Figures 1K and 2), which was significantly reduced compared to WT FMN survival levels and no different from SOD1^{G93A} FMN survival levels. These results indicate that the addition of WT CD4^{+} T cells alone could not restore WT FMN survival levels after axotomy.

Increased non-CD4^{+} naïve peripheral splenocytes, from WT mice, adoptively transferred to SOD1^{G93A} mice unable to support WT levels of axotomized FMN

In an effort to alter the intrinsic peripheral immune compartment of pre-symptomatic SOD1^{G93A} mice and provide a peripheral microenvironment that could potentially activate intrinsic SOD1^{G93A} CD4^{+} T cells after axotomy, WT splenocytes depleted of CD4^{+} T cells were adoptively transferred into SOD1^{G93A} mice prior to axotomy. At 4 weeks post-axotomy, FMN survival levels in the axotomized facial nucleus of SOD1^{G93A} mice that receiv*ed* an adoptive transfer of WT splenocytes depleted of CD4^{+} T cells (SOD1^{G93A} + CD4-depleted WT splenocytes) were 72% ± 4.5, relative to the uninjured facial nucleus (Figures 1L and 2). The FMN survival levels in the SOD1^{G93A} + CD4-depleted WT splenocytes group were comparable to SOD1^{G93A} levels and significantly reduced compared to WT. These results indicate that the addition of WT CD4-depleted splenocytes could not support WT FMN survival levels after axotomy, as observed with the addition of WT unfractionated splenocytes, or prime SOD1^{G93A} CD4^{+} T cells to mediate neuroprotection.

Bypassing the SOD1^{G93A} peripheral microenvironment with adoptive transfer of prior axotomy-activated WT CD4^{+} T cells did not result in WT levels of axotomized FMN

To evaluate whether the addition of prior activated CD4^{+} T cells from axotomized WT mice could bypass the initial activation requirement after axotomy in the SOD1^{G93A} peripheral immune compartment, prior axotomy-activated (*i.e.*, facial nerve antigen-primed; Byram et al., 2004) CD4^{+} T cells were isolated from the cervical lymph nodes and spleens of WT mice after axotomy and adoptively transferred into SOD1^{G93A} mice prior to axotomy. At 4 weeks post-axotomy, FMN survival levels in the axotomized facial nucleus of SOD1^{G93A} mice that received an adoptive transfer of prior axotomy-activated WT CD4^{+} T cells (SOD1^{G93A} + Axot WT CD4^{+} T cells) were 59% ± 5.1, relative to the uninjured facial nucleus (Figures 1M and 2). The SOD1^{G93A} + Axot WT CD4^{+} T cells group exhibited FMN survival levels comparable to SOD1^{G93A} levels and significantly reduced compared to WT levels. Prior activation of WT CD4^{+} T cells was unable to restore SOD1^{G93A} FMN survival levels *via* bypassing the initial activation in the SOD1^{G93A} peripheral immune compartment.

Discussion

Molecular abnormalities in the microenvironment surrounding FMN of $SOD1^{G93A}$ mice are evident prior to and following facial nerve transection (Mesnard et al., 2011). The significant delay in $SOD1^{G93A}$ peripheral nerve regeneration (Mesnard et al., 2013) and reduced FMN survival levels (Mesnard et al., 2011) mirror the response of immunodeficient mice to facial nerve crush or cut axotomy (Serpe et al., 1999; Serpe et al., 2000; Serpe et al., 2002), and implicate a potential defect in immune-mediated neuroprotection. In immunodeficient mice, we have established a crucial role for the $CD4^+$ T cell, initially activated in the peripheral immune compartment, and then reactivated in the CNS by MHCII-expressing glial cells, once FMN have been experimentally disconnected from their target musculature (Byram et al., 2004). Thus, the functionality of $CD4^+$ T cells, peripheral APC, and central glia, is critical to the mechanism underlying immune-mediated neuroprotection.

The objective of this study was to utilize the facial nerve axotomy model to begin to elucidate underlying immune-related defects that contribute to disease in the $SOD1^{G93A}$ mouse model of ALS WT levels of FMN survival after axotomy require functional APC in both peripheral (i.e., B cells, macrophages, or dendritic cells) and central (i.e., microglia) compartments (Byram et al., 2004). If $SOD1^{G93A}$ FMN survival levels after axotomy indeed resemble $RAG2^{-/-}$ mice due to a defect in immune-mediated neuroprotection, this would suggest a defect in either the central glial or peripheral immune response.

Interestingly, our data indicate that WT unfractionated splenocytes adoptively transferred into $SOD1^{G93A}$ mice are able to maintain WT levels of FMN survival after axotomy. These results eliminate the possibility of a defect located centrally in the $SOD1^{G93A}$ mice because the re-activation of the peripherally-activated $CD4^+$ T cell, which is essential for FMN survival (Byram et al., 2004), was able to occur and maintain FMN survival levels to that of WT after axotomy in the $SOD1^{G93A}$ mice when WT mouse splenocytes were adoptively transferred. However, $SOD1^{G93A}$ unfractionated splenocytes adoptively transferred into $RAG2^{-/-}$ mice fail to support WT levels of FMN survival. Yet, isolated $SOD1^{G93A}$ $CD4^+$ T cells are equally as effective in maintaining FMN survival levels as WT $CD4^+$ T cells when adoptively transferred into $RAG2^{-/-}$ mice. Therefore, separation of the $SOD1^{G93A}$ $CD4^+$ T cell from its peripheral microenvironment before adoptive transfer is necessary for the $SOD1^{G93A}$ $CD4^+$ T cell to functionally mediate neuroprotection. However, increasing the number of naïve $CD4^+$ T cells, increasing the number of naïve non-$CD4^+$ splenocytes, or attempting to bypass the intrinsic $SOD1^{G93A}$ peripheral immune compartment with prior activated WT $CD4^+$ T cells all failed to maintain FMN survival levels in $SOD1^{G93A}$ mice when adoptively transferred prior to axotomy. Collectively, these data indicate that a peripheral microenvironment defect exists in the $SOD1^{G93A}$ mouse that may involve the prevention and/or reversal of the appropriate neuroprotective $CD4^+$ T cell development or activation through an active inhibitory mechanism.

Interestingly, polyclonally-activated WT $CD4^+$ T cells, as opposed to naïve $CD4^+$ T cells, are effective in altering disease progression when adoptively transferred to $SOD1^{G93A}$ mice (Banerjee et al., 2008), further suggesting that a defective $SOD1^{G93A}$ peripheral

microenvironment prevents naïve T cell activation. Immunodeficient SOD1^{G93A} mice, crossed onto the RAG2$^{-/-}$ background, possess a shortened life-span (Beers et al., 2008), which can be restored with the addition of WT or SOD1^{G93A} bone marrow, or adoptive transfer of WT or SOD1^{G93A} CD4^{+} T cells (Beers et al., 2008; Beers et al., 2011). Two alternative interpretations of these results are that either the SOD1^{G93A} B and CD4^{+} T cells contribute a degree of neuroprotection in the SOD1^{G93A} mouse model of ALS or the remaining peripheral microenvironment is neurodestructive without functional interactions with CD4^{+} T cells. Both interpretations are supported by the results obtained in the current study. Moreover, the adoptive transfer of anti-CD3-activated WT CD4^{+} T cells into SOD1^{G93A} mice crossed with RAG2$^{-/-}$ mice delays disease progression (Banerjee et al., 2008), as do WT CD4^{+} (Chiu et al., 2008) or CD4^{+}Foxp3^{+} (Beers et al., 2008) T cells. These reports further suggest that exogenous CD4^{+} T cells are capable of mediating neuroprotection in SOD1^{G93A} mice to regulate the ALS disease process, but do not address the functionality of the SOD1^{G93A} CD4^{+} T cells specifically.

In the current study, our novel finding was that SOD1^{G93A} CD4^{+} T cells indeed possess the capability of mediating neuroprotection and supporting MN survival, suggesting that their development is not interrupted but, rather, that their activation is inhibited or disrupted. Therefore, a peripheral splenic microenvironment-mediated defect may underlie the heightened susceptibility of SOD1^{G93A} FMN to axotomy-induced cell death, and a combination of WT CD4^{+} T cells and WT APC is required to overcome the suppressive or dysfunctional SOD1^{G93A} peripheral microenvironment. Collectively, this study demonstrates that the SOD1^{G93A} CD4^{+} T cell is able to respond normally to an antigen-specific response initiated by facial nerve axotomy, but is prevented from functioning in a neuroprotective manner due to a complex immune regulatory mechanism that exists in SOD1^{G93A} mice and modulated only with WT unfractionated splenocytes.

In summary, an overall disrupted homeostatic balance in the peripheral microenvironment (*i.e*., macrophages, dendritic cells, B cells, T cell subsets, *etc*.) and central microenvironment (*i.e*., microglia, astrocytes, infiltrating immune cells, *etc*.) appears to play a role in ALS motor neuron degeneration, disease onset and progression (Troost et al., 1992; O'Reilly et al., 1995; Graves et al., 2004; Henkel et al., 2004; Turner et al., 2004; Rafalowska et al., 2010; Sanagi et al., 2010; Mesnard et al., 2011). Our results point toward a problem in ALS CD4^{+} T cell activation, peripheral antigen presentation, and/or antigen-presenting cell development in the periphery that, in turn, contributes to MN death when disconnection, by disease or experimentally by axotomy, from target musculature occurs.

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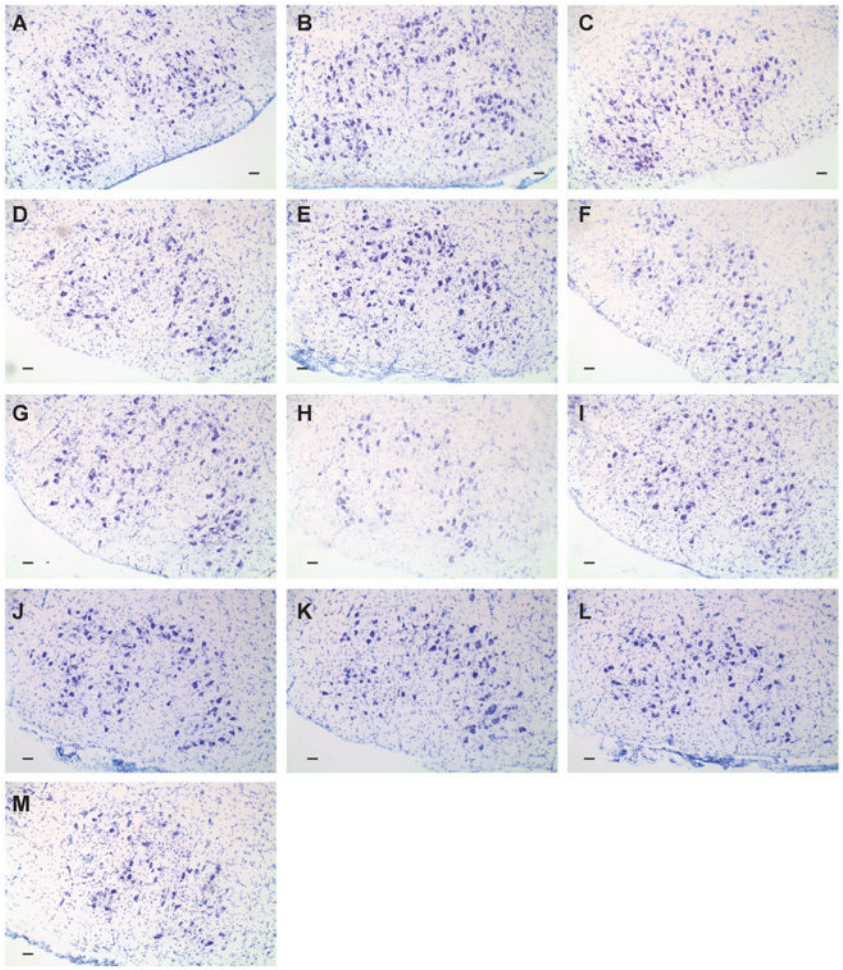

Figure 1. Representative photomicrographs of coronal control or axotomized facial motor nuclei sections from WT, and SOD1^{G93A}, and RAG2$^{-/-}$ mice ± cellular adoptive transfers collected 4 weeks post-axotomy

Representative photomicrographs are shown of control facial nuclei from (A) WT, (B) SOD1^{G93A}, (C) RAG2$^{-/-}$, and of axotomized facial nuclei from (D) WT, (E) SOD1^{G93A}, (F) RAG2$^{-/-}$, (G) SOD1^{G93A} + WT splenocytes, (H) RAG2$^{-/-}$ + SOD^{G93A} splenocytes, (I) RAG2$^{-/-}$ + WT CD4^{+} T cells, (J) RAG2$^{-/-}$ + SOD1^{G93A} CD4^{+} T cells, (K) SOD1^{G93A} + WT CD4^{+} T cells, (L) SOD1^{G93A} + WT CD4-depleted splenocytes, (M) SOD1^{G93A} + prior axotomy-activated WT CD4^{+} T cells. Scale bar indicates 120 μm.

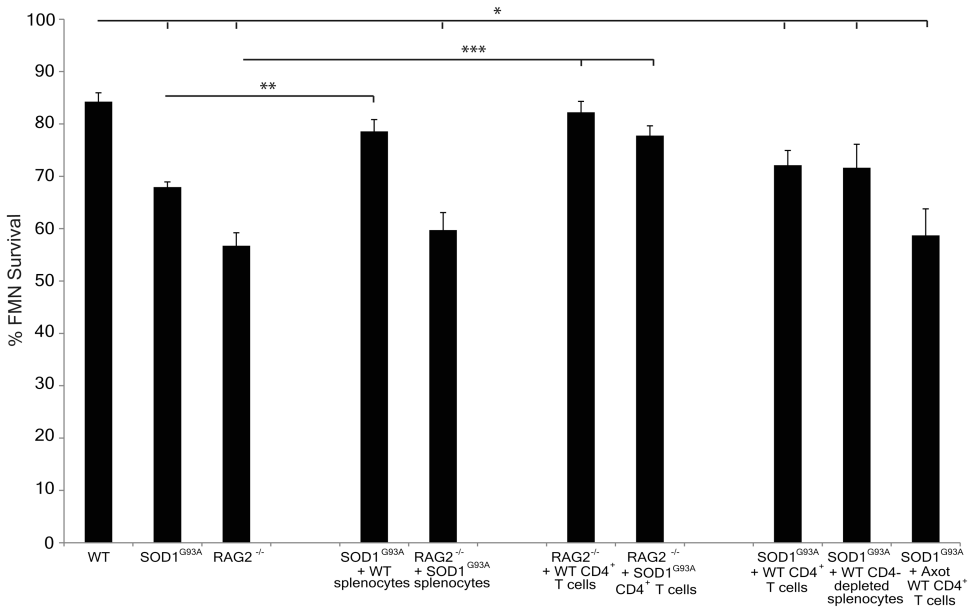

Figure 2. FMN survival in WT, and SOD1^{G93A} and RAG2$^{-/-}$ mice ± cellular adoptive transfers 4 weeks post-axotomy

Average percent survival ± SEM of FMN from axotomized facial nucleus of WT, SOD1^{G93A}, RAG2$^{-/-}$, RAG2$^{-/-}$ + SOD1^{G93A} splenocytes, RAG2$^{-/-}$ + WT CD4^{+} T cells, and RAG2$^{-/-}$ + SOD1^{G93A} CD4^{+} T cells, relative to the uninjured nucleus. Significance denoted by *compared to WT, **compared to SOD1^{G93A}, and ***compared to RAG2$^{-/-}$ at $p < 0.05$.